NITROGEN FIXATION IN LEGUMINOUS PLANTS. VI.

FURTHER OBSERVATIONS ON THE EFFECT OF MOLYBDENUM ON SYMBIOTIC NITROGEN FIXATION.

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(Plate ix; one Text-figure.)

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Introduction.

It is now generally recognized that molybdenum is one of the several "minor" or "trace" elements required for normal growth of plants, and the investigations of recent years have shown that soils more or less deficient in molybdenum occur in several parts of Australia. Such soils were found in South Australia by Anderson (1942), in Tasmania by Stephens and Oertel (1943) and by Fricke (1944), in New South Wales by Shaw *et al.* (1944), and in Western Australia by Teakle (1944). These experimental data refer chiefly to pasture legumes, especially lucerne and subterranean clover; Fricke (1944) states that legumes in field experiments, upon the whole, appear more responsive than grasses to molybdenum fertilizers. Previous experiments (Jensen and Betty, 1943) suggested that molybdenum is needed especially for the process of symbiotic nitrogen fixation in the root nodules of the legumes, as it has long been known to be for the corresponding process of non-symbiotic nitrogen fixation by *Azotobacter* and apparently also certain blue-green algae (Bortels, 1938). The present paper gives some additional evidence derived from experiments which temporarily had to be discontinued owing to the difficulty of obtaining sufficiently molybdenum-free sand for the pot experiments.

Experimental.

In the first series of experiments, lucerne was grown in sand which had already been used for similar cultures until signs of a definite molybdenum-deficiency began to appear (Jensen and Betty, 1943). As in the previous experiments, the plants were grown in glazed earthenware pots holding 3 kgm. of sand with addition of a basal fertilizer mixture consisting of 0·25 gm. K_2HPO_4, 0·25 gm. KH_2PO_4, 0·25 gm. $CaCl_2$ and 0·1 gm. $MgSO_4$. After 150 days, when two cuts of the tops had been taken, the same quantity of salts was again added, together with 3 mgm. of $MnSO_4$, $ZnSO_4$, $CuSO_4$ and $Na_2B_4O_7$ per pot. The experiment included three treatments:

(1). No addition of molybdenum (in the following called "0 Mo").

(2). 1 mgm. Na_2MoO_4 per kgm. of sand ("+ 1 Mo"), added when the first crop was sown (Jensen and Betty, 1943).

(3). 5 mgm. Na_2MoO_4 per kgm. of sand ("+ 5 Mo").

To make up for the removal of molybdenum in the previous crops, each molybdenum pot was given an extra dose of 1 mgm. Na_2MoO_4 per kgm. of sand. Each treatment included six replicate pots with eight plants in each. Lucerne seeds of the variety "Giant Upright", inoculated with an effective strain of *Rhizobium Meliloti*, were sown on 23rd March, 1943. The pots were kept in a greenhouse and watered with distilled water throughout the growth period. The tops were cut four times, after 94, 150, 185 and 216 days. After 150 days, three pots from each treatment were given a supply of combined nitrogen in the form of 0·607 gm. sodium nitrate (= 100 mgm. N) which by analysis was found to contain less than 0·06 part per million of molybdenum.

At the conclusion of the experiment (216 days) the roots were also collected, and the root nodules of the plants that had not received nitrate were separated from the root-substance proper. Nitrogen and molybdenum were then determined in the dried and finely-ground substance of tops, roots, and nodules. The tops of the first two and the last two cuts were bulked for analysis. Nitrogen was determined by the Kjeldahl method, with selenium as a catalyst in the digestion, and molybdenum by Marmoy's thiocyanate method, as described by Piper (1942). All figures are calculated on the basis of material dried at 96–98°C.

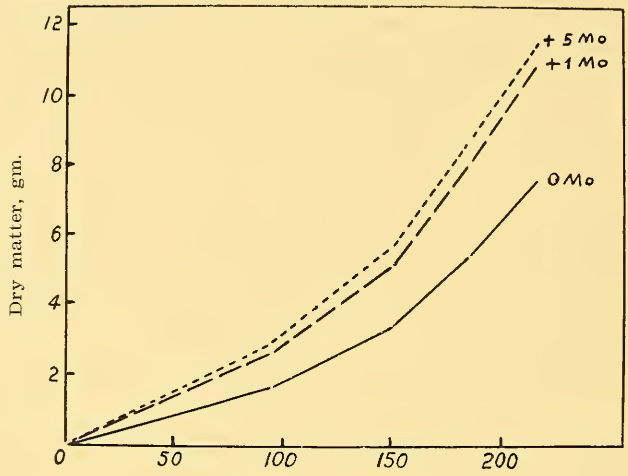

Fig. 1.—Average yield per pot of dry matter in tops of lucerne harvested at four successive stages (94, 150, 185 and 216 days).

TABLE 1.
Influence of Molybdenum on Growth of Lucerne in Sand Culture.

		0 Mo.		1 Mo.		5 Mo.	
		Mean.	S.D.	Mean.	S.D.	Mean.	S.D.
Dry Matter, gm. per Pot.	Tops	7·65	±0·66	10·75	±0·89	11·62	±0·50
	Roots	2·70	±0·32	2·97	±0·13	2·87	±0·23
	Nodules	0·251	±0·032	0·167	±0·035	0·191	±0·066
Nodules in % of Total Dry Matter.		2·38	±0·41	1·19	±0·19	1·29	±0·12
Percentage N in Dry Matter.	Tops, cuts 1+2	3·79		4·48		4·29	
	,, ,, 3+4	3·22		3·34		3·20	
	Roots	1·49		2·84		3·04	
	Nodules	5·02		5·76		5·56	
Total Nitrogen, mgm.		319·0	±19·7	506·0	±41·7	529·0	±26·6
Uptake of N, mgm. per gm. Dry Nodule-substance.		1285	±194	3085	±459	2799	±288
Mo-content of Dry Matter, p.p.m.	Tops, cuts 1+2	0·45		6·0		21·5	
	,, ,, 3+4	0·48		7·8		15·5	
	Roots	0·48		8·6		25·3	
	Nodules	2·7		29·3		62·8	

The yield and composition of the crops (not including the pots receiving nitrate) are shown in Text-fig. 1 and Table 1. The yields of dry matter in tops are seen to be increased by some 40 to 50%, due to the addition of molybdenum, over the treatment "0 Mo", and this influence is fairly constant all through the growth period. The difference between the two doses of molybdenum is not significant. No special symptoms of deficiency were noticed in the plants not given molybdenum, nor was there any visible difference in colour, as observed by Stephens and Oertel (1943) in subterranean clover; the molybdenum-deficient plants merely differed from the others by being smaller in size. The analyses show that the percentage of nitrogen in the tops of the first two cuts is also considerably increased where molybdenum is supplied. As to the roots, their actual weight is not significantly influenced, but the decrease in nitrogen percentage in the molybdenum-deficient plants is even more pronounced in the roots than in the tops, as also found in the previous experiments (Jensen and Betty, 1943). The total yields of nitrogen in the crops therefore show an even stronger effect of molybdenum than do the weights of the tops, being some 60 to 66% higher than in the set of pots without molybdenum, but also, in this respect, the difference between the high and the low dose of molybdenum is insignificant. The nodules show a most interesting phenomenon: not only is there no decrease in the mass of nodule-substance of the molybdenum-deficient plants, but on the contrary, their weight is actually a little higher, and the proportional weight of nodules in percentage of total dry matter is roughly twice as high as in the plants supplied with molybdenum. The increase appears to be due to a larger average size of individual nodules and not to a larger number; although no counts were made, the type of nodule formation looked the same in all three treatments. This stronger development of nodule-tissue and smaller yield of nitrogen indicates a very conspicuous reduction in the nitrogen-fixing efficiency of the nodules in the molybdenum-deficient plants, as shown by calculating the uptake of nitrogen per unit weight of dry nodule-substance; this figure is seen to be nearly 2½ times higher in the plants given molybdenum than in the molybdenum-deficient ones, but again there is no significant difference between the two doses of molybdenum.

The molybdenum determinations show, as in the previous experiments (Jensen and Betty, 1943), that the nodules are constantly richer in molybdenum than the rest of the plants, and this difference is most pronounced in the series "0 Mo" where the plants have still been able to obtain a quantity of approximately 5γ of molybdenum per pot, possibly from the water and the nutrient salts, but perhaps more likely from the gravel that was used for equalizing the weights of the empty pots and as a surface mulch to reduce evaporation. The fact that the tops of the molybdenum-deficient plants contain approximately 0·5 part per million of molybdenum, agrees well with the observation of Stephens and Oertel (1943) that a molybdenum content of about 1 p.p.m. in the tops is necessary for normal growth of white and subterranean clover, and that 0·5 p.p.m. is suboptimal. Furthermore, the present results show that a considerably higher concentration of molybdenum in the nodule-tissue is necessary for an optimal rate of nitrogen fixation. In lucerne, this optimal Mo-concentration seems to lie between approximately 3 and 30 parts per million of dry matter. Most samples of nodule-material taken from plants growing in ordinary soils have shown a molybdenum content of this order of magnitude (Bertrand, 1940; Jensen and Betty, 1943); it would be of great interest to extend these observations to the nodules of plants in molybdenum-deficient soils under field conditions.

The fact that the molybdenum-deficient plants develop a larger proportion of nodule-substance than plants with an adequate supply of molybdenum is interesting in view of the observation of a similar phenomenon in lucerne and clover grown in sand or soil of different reaction, where the relative weight of the nodules tended to decrease in alkaline medium (Jensen, 1943). This might indeed be due to molybdenum rendered available by the addition of lime to the acid substrate (cf., Stephens and Oertel, 1943), but the phenomenon has also been observed, though rarely so markedly, in later experiments where an adequate supply of molybdenum was given. Still another example of the same phenomenon can be found in the data of Jones and Tisdale (1921) on the rate of nodule growth and nitrogen fixation in soy beans at different temperatures. These authors observed that the largest proportion of nodule-substance developed at 21–24°C., but the strongest nitrogen fixation took place at 27–30°C., at which latter range of temperature the uptake of nitrogen (atmospheric plus combined nitrogen from the soil) per unit of dry nodule substance appears roughly twice as high as at 21–24°C. (data from Jones and Tisdale's Tables III and IV).

It thus seems that the legumes possess a mechanism of adaptation by means of which they are able, within certain limits, to compensate for the lower nitrogen-fixing efficiency of the nodules by developing a larger proportion of nodule-tissue in response to unfavourable environmental factors like molybdenum deficiency, acid soil reaction, or suboptimal temperature.

The three pots of each molybdenum-treatment, to which sodium nitrate was added, gave the yields shown in Table 2.

TABLE 2.
Influence of Molybdenum on Growth of Lucerne supplied with Sodium Nitrate.

	0 Mo.	1 Mo.	5 Mo.
	Mean. S.D.	Mean. S.D.	Mean. S.D.
Dry Matter, gm.—			
Tops, cuts 3+4	5·75± 0·52	6·68± 0·37	7·20± 0·05
Roots+Nodules	3·51± 0·14	4·05± 0·23	3·87± 0·32
Percentage N in Dry Matter—			
Tops	2·54	3·17	3·15
Roots+Nodules	1·94	2·09	2·19
Total Nitrogen, mgm.	215·0±16·5	296·0±11·4	310·0± 8·2
(Do. in Pots—$NaNO_3$)	188·0±18·2	285·0± 4·0	287·0±18·8

It appears at once that the increase in weight of tops due to molybdenum is much less pronounced than in the corresponding pots without nitrate, where the combined weights of the third and fourth cut in the three treatments were, respectively, 4·20, 5·72, and 5·93 gm. This levelling effect of the nitrate was most pronounced in the third cut, the first after the addition of nitrate, which gave the following average yields in gm. per pot:

	0 Mo.	1 Mo.	5 Mo.
Pots+$NaNO_3$	2·84	3·44	3·69
% increase due to Mo	—	20	30
Pots−$NaNO_3$	1·94	2·95	3·02
% increase due to Mo	—	52	56

The appearance of the plants immediately before taking the third cut is shown in Plate ix. In the fourth cut, after which all the added nitrate had disappeared from the sand, the effect of the nitrate was still noticeable but much less pronounced; the yields of dry matter in this cut were as follows:

	0 Mo.	1 Mo.	5 Mo.
Pots+$NaNO_3$	2·91	3·24	3·51
% increase due to Mo	—	11	26
Pots−$NaNO_3$	2·26	2·77	2·91
% increase due to Mo	—	23	29

It is further seen in Table 2 that the percentage of nitrogen in the molybdenum-deficient plants is much lower than in the corresponding plants growing with free nitrogen (Table 1). In the plants with the two molybdenum treatments there is a similar reduction in the percentage of nitrogen in the roots (+ nodules), and in all three treatments there is no significant difference in the total nitrogen contents of plants grown with free nitrogen alone and with supply of nitrate.

It thus appears that the production of dry matter under conditions of molybdenum deficiency is less strongly affected when combined nitrogen is supplied than when the plants have to depend on fixed nitrogen only. This, together with the fact that the nodules require for optimal rate of nitrogen fixation a concentration of molybdenum higher than the rest of the plant tissues, seems to show conclusively that molybdenum is essential not only for the general plant metabolism, but also for the specific process of nitrogen fixation. This strongly supports the view that the biochemical mechanism of nitrogen fixation is essentially the same in the leguminous plants and in *Azotobacter*, perhaps also the nitrogen-fixing blue-green algae.

An attempt was made to grow a new (fourth) crop of lucerne in the same sand, this time with addition of sodium nitrate to half the pots from the beginning, but this attempt, unfortunately, failed because the sand appeared in some way to have become unsuitable for further growth of lucerne. The seedlings in this crop showed a heavy mortality, and the surviving plants made only a very poor growth in three months. Further tests were therefore made with two other sands, but these appeared too rich in molybdenum to show any direct response.

The first of these was a faintly acid river sand of medium fineness, containing a fair amount of silt and, according to a previous analysis, 0·02 p.p.m. of molybdenum (Jensen and Betty, 1943). Lucerne was grown in an experiment comprising combinations of the following treatments, in addition to a basal fertilizer mixture similar to the one used in the previous experiment:

(*a*). 0·2% calcium carbonate, to give approximately pH 7.

(*b*). 0·600 gm. ammonium nitrate per pot (= 210 mgm. N).

(*c*). 6·0 mgm. sodium molybdate (2 p.p.m.) per pot.

The first crop of lucerne was sown on 7th March, 1944, in four replicate pots of each treatment, with eight plants in each. This crop grew only very slowly and poorly, agreement between replicate pots was unsatisfactory, and no influence of the molybdenum was discernible when the plants were harvested after 20 weeks. The only significant effect was on the relative weight of the nodule-substance in the pots without ammonium nitrate, which in the acid sand (pH 4·8–5·3) averaged 2·28% of total dry matter, against 1·52% in the alkaline sand (pH 7·0–7·5); the corresponding figures for uptake of nitrogen in mgm. per gm. dry nodule-substance were 1,380 and 2,550 mgm. respectively. The main reason why the experiment is recorded here is that analysis of the crop showed a lower molybdenum content of plants grown with combined nitrogen, as also observed in an earlier experiment in the same sand (Jensen and Betty, 1943). Nodules were practically absent on the plants given ammonium nitrate, and from those with free nitrogen, the amount left over after nitrogen determination was insufficient for separate molybdenum determination. The molybdenum content of the tops and roots is seen in Table 3.

TABLE 3.

Molybdenum Content, p.p.m. of Dry Matter, of Lucerne Plants grown in Sand with Free and Combined Nitrogen.

Addition of Na_2MoO_4		None.		2 p.p.m.	
Source of Nitrogen		N_2.	NH_4NO_3.	N_2.	NH_4NO_3.
Sand $-CaCO_3$	Tops	3·7	0·4	9·5	3·3
(pH 4·8–5·3).	Roots	0·3	(lost)	12·3	7·4
Sand $+CaCO_3$	Tops	0·7	0·7	16·3	10·8
(pH 6·6–7·5).	Roots	4·0	2·0	12·1	15·7

The tendency to reduction in the uptake of molybdenum when combined nitrogen has been provided is obvious, although it is not completely constant, and it is a remarkable fact that the figures for tops and roots are almost reversed in acid and alkaline sand without molybdenum and combined nitrogen. More consistent results were found when molybdenum was determined in lucerne grown for three months in the same sand at two ranges of pH, approximately 5 and 7, with addition of 0·5 p.p.m. of sodium molybdate and combined nitrogen (240 mgm. N) as alternating doses of sodium nitrate and ammonium sulphate. This was one of several experiments, to be discussed in detail later, which were designed to test the influence of varying reaction and nitrogen supply on the nitrogen-fixing efficiency of the root nodules. In this instance the growth of the lucerne was excellent, and sufficient material was available for determination of molybdenum in the nodules, even the small amount of almost ineffective nodule-tissue formed in the presence of combined nitrogen.

TABLE 4.

Molybdenum Content, p.p.m. of Dry Matter, of Lucerne Plants grown with Free and Combined Nitrogen in Sand with Addition of 0·5 p.p.m. Na_2MoO_4.

Source of Nitrogen.	Sand $-CaCO_3$. (pH 5·1–5·4). N_2.	Sand $-CaCO_3$. (pH 5·1–5·4). $NaNO_3$ and $(NH_4)_2SO_4$.	Sand $+CaCO_3$. (pH 7·0–7·4). N_2.	Sand $+CaCO_3$. (pH 7·0–7·4). $NaNO_3$ and $(NH_4)_2SO_4$.
P.p.m. Mo in Tops	22·5	15·4	19·8	19·4
,, ,, ,, Roots	16·3	13·2	21·6	23·5
,, ,, ,, Nodules	136·2	73·7	117·2	93·1

The molybdenum content of this crop is unusually high (a calculation showed that roughly 25% of the added molybdenum had been assimilated), but at acid reaction the higher concentration of molybdenum in plants dependent on free nitrogen is quite unmistakable, especially in the nodule-substance. At neutral reaction there is no such difference, but it is noteworthy that even in plants with such an abnormally high molybdenum content, its accumulation in the nodules is still very marked. There is little difference between the tops and the roots; the tops from acid sand with free nitrogen are even significantly richer in molybdenum than the roots (cf., Table 1, 1st column). The change of reaction produced by the addition of lime has had little influence, except that it has somewhat increased the uptake of molybdenum in the presence of combined nitrogen. Generally the figures form a remarkable contrast to those given by Stephens and Oertel (1943) who found only 2 parts per million of molybdenum in tops of subterranean clover grown in a clay soil with an even higher dose of molybdenum (2·5 mgm. ammonium molybdate per 4 kgm. moist soil) than was used in the present experiment. Apparently the molybdenum-deficient soil used by Stephens and Oertel must have been able to immobilize the added molybdenum.

Incidentally figures like those in Table 4 convey a warning against an indiscriminate use of readily available molybdenum compounds as fertilizers for soils that already contain an adequate supply of this element. The addition of 0·5 mgm. sodium molybdate to each kgm. of sand corresponds roughly to 1 lb. per acre, an amount which is often used under field conditions (Anderson, 1942; Fricke, 1944), and yet the application of this small dose to a sand by no means rich in molybdenum has been sufficient to raise the molybdenum content of the lucerne tops to roughly 20 parts per million. This concentration borders on the limit at which herbage becomes dangerous to domestic animals. Muir (1941), in a discussion of the properties of so-called "teart" pastures in certain areas of England, where an excessive molybdenum content of soil and herbage causes chronic poisoning in grazing cattle, reports that plant material from affected areas contains from 20 to 100 p.p.m. molybdenum in dry matter, against usually less than 5 p.p.m. in healthy areas. He also called attention to the paradoxical fact that "improvement" of such pastures may actually aggravate the condition of cattle, owing to the fact that clovers take up more molybdenum than the grasses, and that application of lime and other basic fertilizers increases the uptake of molybdenum.

After the experiment recorded in Table 3, another crop of lucerne was grown in the alkaline sand without combined nitrogen. This crop, which was sown on 23rd August, 1944, and harvested after 112 days, grew very well, but the addition of molybdenum had no effect on the yields of nitrogen or the efficiency of the nodules, as shown in Table 5. The concentration of 27·6 p.p.m. of molybdenum in the nodules has thus been fully sufficient for their activity (cf., Table 1, treatment "+ 1 Mo"). The only apparent effect of the molybdenum is a somewhat higher percentage of nitrogen in the tops, but this is not sufficient to cause any significant increase in the actual nitrogen content of the crop.

TABLE 5.

Composition of Plants grown in Two Sands with and without Extra Addition of Molybdenum.

Addition of Na_2MoO_4.	None.		2 p.p.m.	
	Mean.	S.D.	Mean.	S.D.
1. Lucerne in River Sand—				
Total N in Plants, mgm.	230	± 32·0	255	± 32·2
Per cent. N in Tops (d.m.) ..	2·40		2·91	
Uptake of N, mgm. per gm. Dry Nodule-substance	2004	±433	2289	±149
Mo in Nodules, p.p.m.	27·6		150·0	
2. Subterranean Clover in Hill Sand, pH 4·8–5·0—				
Total N in Plants, mgm. ..	248	± 57·5	299	± 48·0
Per cent. N in Tops (d.m.) ..	3·05		3·29	
Uptake of N, mgm. per gm. Dry Nodule-substance	882	±135	910	±137
Mo in Nodules, p.p.m.	11·6		37·5	
3. Same, Sand+0·2% $CaCO_3$, pH 7·5–7·8				
Total N in Plants, mgm. ..	282	± 28·2	251	± 27·1
Per cent. N in Tops (d.m.) ..	2·65		3·29	
Uptake of N, mgm. per gm. Dry Nodule-substance	774	± 51·2	915	± 54·5
Mo in Nodules, p.p.m.	20·6		109·0	

Also a fine, yellow, hill sand of acid reaction was tried for possible response to molybdenum. The sand was given doses of 2 p.p.m. of sodium molybdate and 0·2% calcium carbonate, in addition to a basal fertilizer consisting of 1·2 gm. $CaHPO_4$, 0·3 gm. $MgSO_4$, 0·3 gm. KCl, 0·1 gm. $FeCl_3$, and minor elements as in the previous experiments, all per 3 kgm. sand. Subterranean clover, of the variety "Mount Barker", inoculated with effective root-nodule bacteria, was sown in triplicate pots of each treatment, with eight plants in each, on 12th May, 1944, and harvested after 129 days. The results of this experiment are also seen in Table 5. The crop made a very good growth, but the harvest yields do not indicate any beneficial effect of the molybdenum. Its addition has resulted in a somewhat higher percentage of nitrogen in the tops, especially at alkaline reaction, but as in the previous experiment, this is not reflected in a significantly higher return of total nitrogen; at alkaline reaction the weight of the tops was actually somewhat, although not significantly, decreased by the molybdenum addition. The content of 11·6 and 20·6 p.p.m. of molybdenum in the nodules of plants from acid and alkaline sand, respectively, appears to have been fully sufficient; in the alkaline sand the uptake of nitrogen per gm. of dry nodule-substance is actually raised a little by the addition of molybdenum, but although the difference appears significant ($n = 4$, $t = 3{\cdot}267$, P: 0·05–0·02), it is due entirely to depression in the weight of nodules and not to an increase in the yield of fixed nitrogen. It is further seen that in this sand the addition of lime has considerably increased the availability of the molybdenum; not only is the molybdenum content of the nodules approximately doubled at alkaline reaction in the pots not supplied with molybdenum, but the same was the case with the tops which contained 3·0 and 5·3 p.p.m. respectively. The remarkably small increase in molybdenum content of nodules in acid sand plus molybdenum, together with the large increase that results when lime is also added, suggests that the sand possesses some mechanism that renders the added molybdenum unavailable, but that this is counteracted by the addition of lime (cf., Stephens and Oertel, 1943).

No further experiments were undertaken with these sands which evidently contained too large a reserve of molybdenum, but it is hoped to repeat and extend the observations on more molybdenum-deficient growth-media.

Summary.

Pot experiments with lucerne in sand of very low molybdenum content showed a considerable decrease in yield of dry matter and particularly of nitrogen by molybdenum-deficient plants containing only approximately 0·5 part per million of molybdenum in dry matter of tops and roots and 2·7 parts per million in the nodules. Such plants developed a larger mass of nodule-substance than plants with an adequate supply of molybdenum. The gain of nitrogen per unit weight of nodule-substance was approximately 2½ times higher in normal than in molybdenum-deficient nodules. Increasing the molybdenum content of the nodule-tissue beyond 28–30 parts per million had no effect on either the actual or the relative gain of nitrogen. When given a supply of sodium nitrate, molybdenum-deficient plants made a better growth than when dependent on free nitrogen alone; the nitrogen content of the dry matter, however, was greatly reduced. Plants supplied with combined nitrogen generally contained less molybdenum than plants living on free nitrogen; exceptions to this rule were sometimes seen when large doses of molybdenum were given, particularly together with calcium carbonate. An experiment with subterranean clover showed no significant effect when molybdenum was added to a sand from which the root nodules could obtain some 10 to 20 parts per million of molybdenum.

Generally it appears that molybdenum is essential for the specific process of nitrogen fixation, and that the root nodules, in order to carry out this process at an optimal rate, must contain more than 3 parts per million of molybdenum in dry matter, while an increase beyond 20–30 p.p.m. has no additional stimulating effect.

References.

Anderson, A. J., 1942.—Molybdenum Deficiency on a South Australian Ironstone Soil. *J. Aust. Inst. Agric. Sci.*, 8 : 73-75.

Bertrand, D., 1940.—Sur le molybdène des nodosités des légumineuses. *C. R. Acad. Sci. Paris*, 211 : 670-672.

Bortels, H., 1938.—Entwicklung und Stickstoffbindung bestimmter Mikroorganismen in Abhängigkeit von Spurenelementen und vom Wetter. *Ber. deuts. bot. Ges.*, 56 : 153-160.

Fricke, E. F., 1944.—Molybdenum Deficiency. Field Experiments at Cressy, Longford and North Motton. *Tasm. J. Agric.*, 15 : 65-70.

Jensen, H. L., and Betty, R. C., 1943.—Nitrogen Fixation in Leguminous Plants. iii. Proc. Linn. Soc. N.S.W., 68 : 1-8.

————, 1943.—Nitrogen Fixation in Leguminous Plants. iv. Ibid., 68 : 207-220.

Jones, F. R., and Tisdale, W. B., 1921.—Effect of Soil Temperature upon the Development of Nodules on the Roots of Certain Legumes. *J. Agric. Res.*, 22 : 17-32.

Muir, R. W., 1941.—The "Teart" Pastures of Somerset. *Vet. J.*, 97 : 387-400.

Piper, C. S., 1942.—Soil and Plant Analysis. Adelaide.

Shaw, N. H., Barrie, N., and Kipps, E. H., 1944.—The Effect of Lime, Phosphate and Molybdenum on the Growth of Lucerne in Duntroon Loam. *J. Counc. Sci. Industr. Res., Melbourne*, 17 : 233-241.

Stephens, C. G., and Oertel, A. C., 1943.—Responses of Plants to Molybdenum in Pot Experiments on the Cressey Shaley Clay Loam. Ibid., 16 : 69-73.

Teakle, L. J. H., 1944.—Molybdenum as a Fertilizer in Western Australia. *J. Dept. Agric. W. Aust.*, 21 : 335-339.

EXPLANATION OF PLATE IX.

Appearance of lucerne plants in sand with three molybdenum treatments, after 185 days (first crop after addition of combined nitrogen). Above: pots with free nitrogen only. Below: pots with addition of 100 mgm. nitrogen as $NaNO_3$.

(S. Woodward-Smith photos.)